THE SELECTIVE POWER OF TISSUES,

ESPECIALLY AS ILLUSTRATED IN THE MAMMARY GLAND.

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by

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The question of the selective power of tissues, by which they can abstract from the medium in which they are placed those substances which are necessary for their life and function, opens up many problems of fundamental importance in biology. There are points of uniformity between all cells. Every cell respires. Every cell absorbs nutrient material and excretes the indigestible and the effete. But each cell is an independent unit which chooses for itself the substances required for its growth, its development and its functions; and some cells devote their energies to the elaboration of material not for their own individual needs, but for the fulfilment of a function beyond the narrow limits of their own cell-walls.

It is to the study of this selective power that I shall devote my thesis. No tissue of the body seems to me to possess this prophetic power of selection in so eminent a degree as the Mammary Gland, engaged as it is in the elaboration of a material adapted to the requirements not of its ~~won~~ own cells, not of the organism of which it forms a part, but of an entirely different organism. I propose to enter, after describing some of the most striking phenomena of selective activity as exemplified in other tissues into the subject of the Chemistry of the Mammary Gland and its secretion, with special reference to the production, in different species, of milk suited for the diverse needs of the sucklings of these species. The work on the Mammary gland and much of that on the chemistry of milk has been carried out by me in the Physiological Laboratory of Edinburgh University. As for the rest I hope to submit a digest and a criticism of the work of others, some of which may not be widely known, and all, I trust, will be of Physiological interest and practical importance.

<u>The selective power of the Cell.</u>

The power of selection is illustrated in its most elementary form in the case of unicellular organisms. Some of the minute sea organisms build up a skeleton of carbonate of lime, others of silicious material, and many of these can live and thrive together in one community of sea water, for the salts the surrounding medium contains are many, and this one requires one salt, that one another. Are not the strata of chalk, the beds of silicious matter, the islands of coral all monuments to this selective power, - great results of the minute forces exercised in a single animal cell. Plants, both marine and terrestrial, show most clearly that they select from the plenitude of material offered to them those substances necessary for their life and their growth. Surrounded as they are by a fluid very rich in Sodium chloride, marine plants absorb and build up into their tissues very little of it, while they take up relatively large amounts of Calcium, of Potassium or of Iodine, of which only traces are present.

In the animal body the activity of the cells is governed by the same great law. Some groups of cells take up Calcium salts from the small quantities present in the blood and deposit them in the bones; some store up Iron to form haemoglobin and fit them for their work as carriers of oxygen; some, providing for future needs, take up food materials and store them until a need for them arises; and some remove from the blood effete matters and excrete them in the bile and in the urine. All are working to a great end - the good of the whole, each in its own special sphere, each with its own peculiar duty.

In some cases the selective power of the cell seems to depend on the satisfaction of a chemical affinity, and on this depends many of the staining phenomena employed for the study of morphology. Ehrlich's intra vitam methylene blue method is dependent on the affinity this stain has for nerve fibres when injected into the living animal. Heidenhain's experiments on the secretion of the kidney depend on the cells of the epithelium lining the convoluted tubules being stained by indigocarmine in an effort to excrete that substance from the blood, and the beautiful differential stains for nuclei and protoplasm with the anilin dyes rest on the chemical affinities of these structures for basic or acid dyes. The basic anilin dyes have a strong affinity for chromatin, the acid dyes more especially for the protoplasmic structures of the cell; and on this fact Lilienfeld built up the theory that the various members of the nuclein series show an affinity for basic dyes in proportion to the amount of nucleic acid they contain. Malfatti tested this by staining a series of proteids from pure albumin containing no phosphorus, through the nucleo-albumins such as caseinogen which contain a small proportion of phosphorus to the nuclei of Spermatozoa consisting of nearly pure nucleic acid. He employed a mixture of red acid fuchsin and basic methyl green, and found that while pure albumin was stained red, and the spermatozoon head intense green, the nucleo-albumins took on reddish green or purple hues varying according to the amount of phosphorus present. The correctness of this test has since been denied, but it offers a tempting method of studying the phenomena occurring in the cell, and the relation of the nucleus to the protoplasm during rest and during active change. Certain it is that the staining power of chromatin is greatest when in the preparatory stages of mitosis, and diminishes during the resting period of the nucleus suggesting that a combination between the nucleic acid of the chromatin and albumin has taken place. Wilson, in his admirable treatise on the cell, discusses this question fully, and suggests the hypothesis, based on the work of Kossel and others on these reactions of the nucleus, that "nuclein is, in a chemical sense, the formative centre of the cell, attracting to it the food matters, entering into loose combination with them, and giving them off to the cytoplasm in an elaborated form". The work of Rückert on the ova of the shark is of great interest in this connection. At a very early stage in the life of the egg the chromosomes are small, and stain intensely with nuclear dyes. As the egg grows they increase greatly in size, but step by step with the increase there is a diminution in their staining power. As the egg approaches full size the chromosomes again diminish in size and regain their staining power. It is suggested that during the period of rapid growth of the egg the nuclein of the chromosomes combines with albumen, and, the proportion of phosphorus in them being thus diminished, their staining power with nuclear dyes also decreases. Then finally they give off matter to the surrounding protoplasm as food elaborated for the needs of the protoplasm, and resume their highly phosphorised and deep staining properties. This remarkable series of alterations in the affinity of the nucleus for dyes points to an intimate connection between the nucleus and protoplasm as regards the selection and elaboration of food material for the cell.

Next to chemical affinity the physical process of osmosis seems to be of importance in the absorption of material into the cell. Osmosis is a result of the attraction between the molecules of fluids on either side of a permeable membrane; and of the attraction the membrane has for the two fluids. The cell wall is the permeable membrane. On the one side is the fluid in the meshes of the protoplasm, on the other side is a fluid holding food-material in solution. Two streams are set up through the membrane. The one entering the cell conveys the material which is to be employed by the cell; the opposite stream carries out effete matter. All substances do not diffuse through a permeable membrane with equal readiness, and, although it is possible that absolutely useless or even hurtful material may enter the cell, yet it is equally possible that this physical process plays an important part in, as as it were, granting or denying permission to solutions to enter. In the same way it has the power of retaining in the cell-sap substances which are of use to the cell, and which would otherwise be washed out by the fluid bathing the cell.

Other physical and chemical phenomena play a part in the formative work of the cell. A certain amount of heat is necessary that the metabolic processes may go on. Carbon dioxide is not taken up by plants in the dark, the presence of sunlight is necessary. The entrance of foreign substances into the cell may diminish its vitality and hinder its metabolism, and many peculiar tricks may be played on the division of ova by the chemical stimuli of dilute poisons, and by abnormal physical conditions.

The selection by the tissues of the animal body of substances required for their development is illustrated ab initio, from the time when the germ cell begins its growth. In the lowest forms of multicellular organisms there are two kinds of cells. The smaller are for nourishment and locomotion, the larger for reproduction, and all the others seem subservient to those which have the higher destiny to fulfil—namely the carrying on of the race. In the higher types there are special germ cells set apart, supported and nourished by cells whose duty it is to support and nourish them. The history of the ovarian development of the egg is a record of the changes involved in its nutrition and the storage of nutritive material. The young ovum is to a large extent relieved of the burden of choosing and elaborating food for itself. In some of the insecta each ovum is accompanied by a nurse cell. This nurse cell is at first much larger than the ovum itself. It contains a large nucleus rich in chromatin, and the egg grows at the expense of the nurse which shrivel~~l~~s, loses its chromatin and finally disappears. In the earthworm several eggs are laid, only one of which grows by feeding on its sister cells. And in higher types several cells derived from the same epithelium are set apart. To one of these is appointed the destiny of becoming the means of reproduction, while the others resolve themselves into a protecting and commissariat department for the ovum.

The female germ cell supplies most of the material for the body of the embryo and stores the food by which it is to be nourished in its early stages. The changes which take place in the ovum during maturation are all prophetic. Thus it is devoted to the accumulation of protoplasm and deutoplasm, and its nutritive processes are largely anabolic or constructive.

In the very young ovum the amount of protoplasm is small but as it enlarges there is a great increase in the protoplasm and this is accompanied by the appearance of nutritive yolk granules or deutoplasm. The origin of this deutoplasm is doubtful. In some animals it has been said to appear first at the periphery of the cell and then to advance towards the nucleus. In others it is said to appear in the protoplasm of the cell like zymogen granules in gland cells. In some cases it is said to consist of parts split off from the nucleus and these have been described as actual buds. Whatever its origin may be, its function, doubtless, is the nutrition of the protoplasm of the cell. The observations of Rückert on shark ova point to the importance of the activity of the nucleus on the formation of the cytoplasm of the ovum, and it would seem probable that the nucleic acid of the nucleus attracts food substances which enter into the cell sap, elaborates these and gives them off to the protoplasm as fully formed foods. Kossel comes to the conclusion that the formation of new organic matter is dependent on the nucleus, and that the phosphorus-holding nuclein plays an important part in the process. If a unicellular organism be cut in two, the nucleated portion repairs the wound and goes on living, digesting, secreting. But the non-nucleated portion, though it may live for several days, does not heal its wound and loses the power of digestion and secretion. Thus it would appear that the function of the nucleus in the elaboration of protoplasm is an important one. There is no doubt that the nucleolus, which is less highly phosphorised than the chromatin of the nucleus, and a large part of the germinal vesicle are thrown off into the protoplasm of the ovum at the time of maturation.

The male germ cell supplies little if any protoplasm to the ovum. It consists only of nuclear material, a centrosome, and just enough protoplasm to supply energy for its active movements of locomotion. The series of changes in the ovum during maturation are all directed towards the preparation of the cell for growth and division. The disappearance of the centrosome prevents the possibility of parthenogenesis; the extrusion of the polar bodies diminishes by one half the number of centrosomes, and prepares the cell for the reception of the nuclear material of the spermatozoon head, and for the formation of a nucleus partaking of equivalent parts of paternal and maternal characteristics. From the state of quiescence of the mature egg the entrance of the male centrosome starts the wonderful minute forces of the cell into an activity not to cease until the death of the organism. Before the arrival of the spermatozoon the ovum, or its nurse cells have been engaged in the selection of food material adapted for the building up of the protoplasm of the ovum, and although the ovum supplies nearly all the protoplasm for the embryonic body, yet the spermatozoon is as potent in its influence on the offspring as the female germ cell. This in itself is sufficient proof that the nucleus is all important in ordering the conditions of growth of the organic matter in the cell. It seems certain, also, that it is on the phosphorus-rich nuclein that this power depends, and phosphorus seems to play as important a rôle in the constructive phase of metabolism, as iron does in the carriage of oxygen.

The nutrition of the embryo.

The ovum contains within itself the food substance necessary to provide energy for the earlier processes of division which result in the formation of the embryo. There is a great difference as regards the supply of food material between the holoblastic ovum of the mammal and the meroblastic ovum of the bird. In the latter case a large quantity of nutritive substance is prepared before division has begun and this forms the only source of nourishment for the young creature until the end of incubation. In the holoblastic egg there is only sufficient foodstuff for the first few steps, but a yolk-sac is formed which becomes filled with the necessary food apparently absorbed from the fluids in which the ovum is bathed, and thus development is carried on to a further stage. A fluid appears among the cells of the so-called "mulberry mass" leaving a layer which becomes flattened against the inner side of the zona pellucida and pushing to one side the cells which become differentiated into the three primary layers of the embryo. The cells of the hypoblast grow and spread so as to line the ovum all round and thus a cavity filled with fluid is formed which is termed the yolk sac. In birds this sac contains the highly nutritive yolk, and in holoblastic ova the fluid in the yolk-sac is doubtless of a similar kind. The material of which it is composed is absorbed from the outside, and the duty of selecting a suitable nutritive material belongs to the clear cells which line the original vitelline membrane. By the bending over of the visceral plates a part of this blastodermic cavity is separated off to become the primitive alimentary canal, and the hypoblast lining the intestine is therefore continuous with the lining membrane of the yolk-sac. Numerous bloodvessels spreading out on the surface of the yolk-sac or umbilical vesicle absorb the food material it contains. In mammalia the office of the umbilical vesicle ceases at a very early period as the quantity of the yolk is small and the embryo soon becomes independent of it by the connections it forms with the parent. In birds these blood-vessels are of course larger and as the sac is emptied it is drawn within the abdomen whose walls close over it. In mammals it remains on the outside of the umbilicus and the remains of it may be seen even after birth, a relic of the early processes of nutrition.

The composition of yolk of egg is interesting as an analogy may be drawn between it and the milk with which the young mammal is nourished after birth. Both are foods selected for the same end - their suitability for the nutrition of a growing organism. An analysis of the yolk of the hen's egg gives approximately the following figures:-

Water	50.82	
Nitrogenous Substance	16.24	
Fat	31.75	
Salts	1.09	(König)

The proportion of salts to one another in the ash of the yolk and in the ash of milk is as follows:-

	In 100 parts ash of yolk.	In 100 parts ash of human milk.
K_2O	8.5	33.7
Na_2O	5.3	9.9
CaO	12.0	14.1
MgO	1.9	2.9
Fe_2O_3	1.3	0.19
P_2O_5	59.9	20.4
Cl	11.0	18.8

The analogy between the two is of course not perfect as the yolk is a food supplied for the nourishment of the chick at a much earlier period of development than the milk is for the young mammal. The conditions are vastly different. The rapidity of growth is much greater in the yolkfed chick than in the milkfed child. But still an analogy exists and it is interesting to note the points of resemblance and the points of difference. The most striking difference is in the amount of phosphorus. The yolk contains a much greater amount of this element and the greater part of it is organically bound. This is important having regard to the hypothesis that phosphorus is essential as food for growing cells. The caseinogen of milk and the vitellin of yolk of egg are the only two nucleo-albuminous compounds present to any considerable extent in food-stuffs, and it appears to be indubitable that these phosphorised foods are of especial importance for the upbuilding of the growing tissues. And the reason of the greater richness in phosphorus in the yolk is that it is a food suited for the nutrition of more rapidly growing tissues. It is probable that the food material in the yolk-sac of the young mammal is of much the same nature as that of the yolk of the chick with less salts perhaps, but rich in nitrogen and in phosphorus.

The nutrition of the foetus.

A stage later in development when the food-supply of the umbilical vesicle is exhausted, the seat of absorption of the constituents of food ~~for~~

for the foetus is changed, and this duty devolves first on the chorionic villi and then on the placenta. When the allantois, growing from the hinder end of the embryonic intestinal canal, reaches the outer wall of the ovum, the whole surface becomes covered by shaggy outgrowths. The allantois becomes very vascular. Bloodvessels are formed in the chorionic villi, and a vascular communication is formed between the foetus and the chorion. After impregnation the mucous membrane of the uterus thickens, its follicles enlarge and the vascularity of the whole mucous membrane is rapidly increased. The cavity of the uterus becomes filled with fluid secreted from the uterine glands, and into this fluid the ovum comes on its entrance into the uterus. Probably the fluid is nutritive and the function of the chorionic villi is to absorb it for the nourishment of the foetus. It appears probable then that the epithelial cells of the uterine glands take on the function of selecting a food material suited for the foetus from the bloodvessels in the very vascular uterine mucous membrane.

This mode of nutrition also passes and the foetus becomes anchored to the uterus of the mother by the placenta. The villi of the chorion dip into sinuses formed in the deeper part of the mucous membrane of the uterus and the foetal and maternal bloodvessels come into close relation to one another. There is no direct communication between the bloodvessels of the mother and those of the foetus but one or more layers of cells separate the blood of the one from that of the other. The question whether one or more layers of nucleated cells exist in this position has been much discussed and is still an open one, but from analogy it would appear that, during all the time when the young creature is deriving its food supply from the mother, both before birth and during lactation, the duty of selecting the food material for the child belongs to the tissues of the mother. Before the period of placental life and again after birth the tissues of the mother select the food, and it would be remarkable if at this period in the life of the foetus that duty belonged to the foetal cells covering the villi. It has been stated and again denied that a space, bounded on the one side by maternal and on the other by foetal cells, exists, and it would be more in line with what we know of the processes of nutrition during the remainder of the period of development if this were actually the case.

The blood on the foetal side of the placenta is not identical with that on the maternal side.

Winternitz, in a paper on the blood of newborn animals points out that the blood of a healthy newborn animal is richer in blood colouring matters than at any later period of life. This richness in haemoglobin falls rapidly in the first week and then more slowly until in the 10th or 12th week it comes to be equal to that of adult. Not only is this true of blood-pigment, but also of total dry substance.

It seems, therefore, certain that the placenta has the power of selecting the food constituents necessary for the foetus from the mother's blood and of sending a food properly elaborated into the vascular system of the foetus. The placenta possesses the additional function of excreting from the foetal blood the effete material it contains. The blood which may be expressed from the placenta is very rich in urea. The removal of urea from the blood in the kidneys is not a process of diffusion but a true secretive action, the work of the cells of the convoluted tubules, and it is no less likely that the cells in the villi of the placenta remove the excess of this substance from the blood of the foetus by a similar secretive action.

The history of the nutrition of the developing organism is a remarkable one and passes through various phases. In all phases the same thing is true, that it is the duty of certain cells to select a suitable food material, from the fluids of the mother's body, and after birth the child continues to feed on the blood of its mother, but the seat of selective activity is changed to the mammary gland. The secretion of milk is therefore a matter of great physiological importance, and, although the changes in the gland cells during rest and during activity have been very largely studied from the histological standpoint, little attention has been given to the chemistry of the secreting gland. Before entering into a description of the gland from this point of view I propose to discuss some points of interest which suggest themselves with regard to the constituents of milk.

Milk, besides playing a great part in the foods of civilised and uncivilised peoples is a food adapted by nature for the needs of the suckling. The measure of its difference in different animals is the measure of the difference between the physiological needs of the young animals of these types. Let me in the first place draw a comparison between the diet appointed for the growing child and the freely chosen diet of the adult. In a freely chosen diet the foodstuffs are composed in proportions varying within certain limits and under certain conditions, of three chief kinds of organic substance - proteids, fats and carbohydrates, together with salts and water. Many experiments giving widely different results have been carried out in order to ascertain the quantities of these primary alimentary principles necessary for the support of an adult organism, and Voit and Pettenkofer give the following quantities as requisite for an adult man weighing 70 kilograms.

	Resting	Working.
Proteid	137 grams.	137 grams
Fat	72 "	173 "
Carbohydrate	352 "	352 - 500 grams.

The amount of Salts taken daily by an adult is stated in Ranke's diet scale to be 25 to 28 grams. More recently it has been stated that the amount of proteid absolutely indispensable for man is much less than the amount quoted above, and some observers give the amount of carbohydrate required by a man during a considerable amount of work as higher than was deemed sufficient by Voit. Feer and Camerer and Söldner have described experiments made on the feeding of normal infants, and they conclude that a healthy child in about the 30th week, and weighing seven kilograms, takes about 1000 C.C. of milk daily. It will be of interest to ascertain how much of each constituent is taken in such a diet, and not merely of scientific interest but it may be of importance with a view to the preparation of a rational artificial food for sucklings.

The percentage amount of proteid in woman's milk has been very differently stated, partly of course on account of actual variation in the specimens examined but partly also on account of errors in the methods of earlier investigators. Many tables of Analyses of milk are given in works on Physiological Chemistry by Bunge, Gorup-Bésanez and others, in König's volumes on the human food stuffs, and in the scattered papers of physiologists, physicians and veterinary scientists. The results of these vary very considerably not only in proteids but also in the carbohydrates, Fats and Salts of milk. Taking as the average composition of human milk the following figures, we can easily ascertain the amounts of each alimentary constituent taken by the suckling in its food.

Proteid	1.86%
Fat	3.48"
Carbohydrate	4.36"
Ash	0.21"

Therefore a healthy child of about 30 weeks, weighing seven kilograms, and consuming 1 litre of milk per diem takes daily the following amounts of these constituents:-

Proteid	18.6	grams
Fats	34.8	"
Carbohydrate	43.6	"
Salts	2.1	"

The freely chosen diet of an adult bears a remarkable comparison to the diet selected by the cells of the mammary gland to meet the requirements of the young child. If the child of seven kilograms weight were to take its food constituents in the same ratio as the adult of 70 kilograms does in proprtion to its body weight, it would daily receive:-

Proteid	13.7	grams
Fat	7.2	"
Carbohydrate	35.2-50	"
Salts	2.5	"

Thus it would appear that per kilogram of body weight the milk-fed child takes more proteid and more fat than an adult and less carbohydrate than a man doing a fair amount of external work; and it is at first sight very remarkable that the amount of salts taken by a child seems to be less than that consumed by an adult. In the adult the anabolic process is as nearly as possible equal to the katabolic;

in the child the former greatly exceeds the latter, and the needs of the suckling must therefore differ widely from the requirements of the adult. The difference may be stated as follows:-

I. The suckling requires more proteid because it requires much nitrogenous matter for the building up of its tissues, a process which goes on during the period in which milk is the sole food, with remarkable rapidity. The adult has only his existing tissues to maintain. Many authors have denied that the adult organism has any need for so much even as 100 grams of proteid daily. Hirschfeld found that he could greatly diminish the amount of proteid taken in his food while maintaining perfect health, but in order to do this he had to greatly increase the amount of Carbohydrate. Kumagawa sought to prove, by investigations into the diet of the Japanese who live principally upon rice, that so much proteid as Voit and the earlier writers on the subject, stated, was unnecessary. The large amount of proteid in the diet of the suckling is astonishing, even when the excess of anabolism over katabolism is taken into account; and it appears to support the earlier writers who asserted that more than 100 grams of proteid per day was necessary for an adult. Not only is the need for nitrogenous material great in the young animal, but great also is the need for phosphorised material with which the nuclei of the rapidly multiplying cells of the tissues may be built up. If the hypothesis stated earlier in this paper be a correct one, then phosphorus in organic combination is an essential for the multiplication of cells and the growth of protoplasm; and some support is given to the hypothesis by the fact that of the nitrogenous material supplied to the growing organism in the milk, the greater part of it is in the form of a nucleo-albumin. It is also apparent that a lactating woman who has to part with such a large amount of proteid daily has a great tax put upon her metabolism, and must require in her diet much more proteid than one who has not such a large proteid-bill to meet daily.

II. The suckling requires more fat as heat material, since it has not to such a large extent as the adult the muscular source of heat supply. The evolution of heat goes on in the muscles at rest as well as during their periods of activity, but the proportion of muscular tissue in the body of the young child is not nearly so great as in the adult body. The young child has also a relatively greater body surface from which the body heat may be dissipated.

In addition the young child has a higher body temperature to maintain than the adult - the difference being almost 1° Fahrenheit. All these are in face of the fact that constructive metabolism, which so greatly exceeds katabolism in the growing body of the young child, is unaccompanied by the evolution of heat. For these reasons a larger proportion of heat-producing food is necessary.

III. The adult requires more carbohydrate because he performs more work. The external work performed by the young child is exceedingly small in amount. The energy employed for the performance of work can undoubtedly be supplied by the oxidation of all organic matter, but it seems certain that carbohydrates play an important part in providing this energy. Muscle can build up its bioplasm only out of proteid, but the mechanical energy directed to the performance of muscular work is not necessarily a result of the combustion of the muscle substance itself but of organic material present in the muscle. Assuming that, in an adult, carbohydrate is a source of mechanical energy, then so large an amount of that substance will not be essential in the food of the young child. But the difference is not so striking as might be expected. The milk-fed child actually gets more carbohydrate per kilogram of its bodyweight than an adult in a state of rest, though less than is necessary for an adult performing a considerable amount of work. But the amount of energy and the source of it required in the work of nutrition and growth are unknown quantities.

lV. The suckling requires more salts than the adult for the building up of its tissues particularly for the formation of its bones. But, although it is to be expected that the young animal whose cartilage is being rapidly replaced by bone rich in salts would require a much larger proportion of salts than the adult, yet per kilogram of body weight, there is no remarkable difference. Indeed the average adult takes per kilo more salts than the suckling. According to Ranke's diet scale an adult takes in his food about 25 grams of salts daily, whereas a child of one tenth the body weight takes, in one litre of human milk only 2.1 grams per diem. The explanation of this is not far to seek. It seems to be the case that an adult on a normal diet exceeds his requirements in the matter of salts, or he finds a use for the excess of salts unknown in the economy of the growing child. "It is probable", says Bunge, "that the adult organism could exist with a very small amount of salts: it is a priori difficult to see what the constant addition of salts is for". This constant addition of salts to the ordinary diet of an adult consists entirely of Chloride of Sodium, which enormously exceeds in amount the other salts taken. Most people take more than 20 grams of common salt daily. There are other salts as important in the economy of the tissues as common salt, yet it is the only one chosen and consumed at nearly every meal. Bunge tries to find reasons for this, and concludes that in the first place it is required to counteract the effect which potassium salts, so richly provided in vegetable food have on the sodium chloride in the blood. A partial exchange takes place between carbonate of potassium and chloride of sodium, and thus the total amount of the latter which is the chief salt of the blood-plasma would be diminished unless the diminution were prevented by the taking of sodium chloride. In the second place common salt is taken as a mere condiment. The suckling, having no vegetable food, has therefore not the same need for this salt as the adult, and to him condiments are unknown. The total amount of sodium in milk is very small, indeed in the 2.1 grams of salts present in a litre of human milk, only .23 grams are sodium salts. In comparing the food of the adult with that of the suckling in the matter of salts we must consider only the formative salts, those which are required for the growth of the tissues. If we eliminate sodium salts from our calculations we may rightly conclude that the amount of the other inorganic salts in the natural diet of the growing child far exceeds, per kilogram of body weight, the amount of the same salts in the diet of an adult. And this is only what one would expect. Excluding Sodium chloride an adult certainly takes no more than 5 grams of salts, whereas a suckling with but one tenth the body weight takes about 2 grams. With the single exception of yolk of egg milk is by far the richest of our foods in bone-forming calcium salts, and it must be remembered that yolk of egg is in the same category of foods as milk, namely a food selected as suitable for the rapidly growing tissues of a young animal - an animal indeed which at the time of hatching has its bones in a more forward state of development than are those of a young mammal at the time of birth.

The Milk of different Animals.

The composition of the milk of various animals is astonishingly different. Many analyses of different kinds of milk have been made and brought together in physiological textbooks, and these analyses reveal differences in the nature and conditions of the animals for which the milk is provided. It is at least plausible that the mammary glands of various animals select the constituents of milk in proportions adapted to the diverse conditions of life under which those animals are placed. Equally with the variations in the conditions of environment, of activity, of growth, which influence the suckling, so varies the composition of the milk provided for its needs.

The following propositions may be submitted and the grounds on which they rest investigated.

1. Animals which grow rapidly are supplied with milk containing more of those constituents which prevail for the building up of the tissues and organs than animals which grow more slowly. These constituents are proteids and salts.

2. Animals which live under different conditions of temperature are provided with milk containing constituents in proportions more or less useful for the maintenance of body heat.

3. Animals which are compelled to immediate muscular exertion receive milk adapted to supply the materials for the production of muscular energy.

1. Bunge, whose work is wonderful for its suggestiveness, has made a comparison between the salts of the milk and those contained in the whole body of the young animal for whose nourishment the milk is

provided, and has elicited the remarkable fact that the proportion of the various inorganic substances to each other in the milk, is almost the same as it is in the whole body of the new-born animal. From the point of view of the mammary gland as a selector and elaborator of the food materials in the milk this correspondence is all the more remarkable as the composition of the ash of blood serum, from which the food materials are abstracted by the cells of the mammary gland, is very different; and, as I propose to show, it differs also considerably though not so much from the quantitative composition of the ash of the mammary gland itself. An analysis of the ash of a human suckling for comparison with the composition of the ash of human milk has not been made, but there is no reason for supposing that the same remarkable correspondence does not hold good in the human species as well as in the case of the dog, in which animal the investigation was made.

One hundred parts by weight of ash contain:-

	In Newborn dog.	In dog's milk.
K_2O	8.5	10.7
Na_2O	8.2	6.1
CaO	35.8	34.4
MgO	1.6	1.5
Fe_2O_3	0.34	0.14

	In Newborn dog.	In dog's milk.
P_2O_5	39.8	37.5
Cl.	7.3	7.1

The milk, therefore, as regards the individual salts present in it, is peculiarly adapted for the maintenance of the normal proportion of salts present in the suckling. At present I leave over the discussion of the individual salts.

It appears from an early series of milk analyses by Bunge that the total ash in the milk of various animals varies directly with the rapidity of growth in the young of these animals. He gives the following amounts of ash as occurring in the milk of the animals mentioned:-

	Ash in 1000 parts milk.
Man	2.2
Horse	4.1
Cow	8.0
Dog	13.1

Man certainly grows slower than the horse, horse than cow, cow than dog. Pröscher tried to prove the correctness of this statement in a great number of animals. He brought together a series of analyses of milk made by various investigators and added some himself, and, weighing the young animals of those types in which it was possible, - fed on their own mother's milk - at regular intervals ascertained the time required by them to double their weight. Let me give a table showing the total amounts of ash in the milk of various animals compiled from various sources, and alongside of them the dates of the doubling of the weights of the sucklings in such cases as it is possible to obtain them.

	Ash	in 1000 grm Milk	Time of Doubling Weight (Pröscher)
Hippopotamus.	1.0	(Textbook of Physiology) ed. Schäfer)	
Man	2.2	(Bunge)	180 days.
Ass	3.0	(Schäfer's Textbook)	
Horse	4.3	(Konig)	60 "
Goat	6.2	(Group-Besanez)	
Elephant	6.5	(Konig)	
Camel	7.7	(do)	
Cow	8.0	(Bunge)	47 "
Buffalo	9.7	(Schäfer's Textbook)	
Pig	10.5	(do)	18 "
Sheep	11.9	(do)	10 "
Dog	13.1	(Bunge)	8 "
Rabbit	25.6	(Pröscher)	

The history of the various animals mentioned above, as regards their rate of growth is written in the chemical composition of the milk of their species, and it is almost impossible to say that we could fill up the gaps in the latter table by our knowledge of the amount of salts present in their milk. Thus easily is the selective power of the mammary gland demonstrated.

No less remarkable is the correspondence between the amount of nitrogen-holding bodies in the milk and the rate of growth of the young creatures and it will be seen from the table given below that these substances go hand in hand with the salts of milk having regard to the rate of growth of the sucklings. The nitrogen-holding bodies in milk are almost all also highly phosphorised, and the importance of this fact has also been remarked.

	Nitrogenous Substances in 100 parts Milk.	Date of doubling weight.
Man	1.86	180 days
Ass	2.2	
Horse	2.33	60 days
Goat	2.8	
Elephant	2.95	
Camel	3.67	
Cow	4.0	47 days
Buffalo	5.7	
Pig	6.98	18 days
Sheep	7.0	10 days
Dog	8.28	8 days
Cat	9.52	5 days
Rabbit	15.54	

It will be seen that the order of animals in richness of proteid substance is identical with that in richness of salts.

This law, so admirably illustrated by those tables, in the development of different species, seems also to be valid for the development of the individual.

The milk during the first few days after parturition is extraordinarily rich in solid substances, and from that time to the end of lactation it gradually loses in proteid and in fat, while an increase in milk-sugar and water keep pace with that diminution. Pfeiffer gives the following results of analyses of milk during the early days of lactation.

In 100 grams Milk.

<table>
<tr><th></th><th>Proteid</th><th>Fat</th><th>Sugar</th><th>Ash</th></tr>
<tr><td>1-2 days after parturition</td><td>8.6</td><td>2.4</td><td>3.1</td><td>0.37</td></tr>
<tr><td>3-7 " " "</td><td>3.4</td><td rowspan="2">3.1</td><td rowspan="2">5.4</td><td rowspan="2">0.26</td></tr>
<tr><td>8-14 " " "</td><td>2.5</td></tr>
</table>

After 14 days we find the proteid percentage fallen to 2.5. At or about this level it remains during the first two or three months and then it falls in the second half year to even less than 1%. At the same time the fat according to the tables of analyses given by König falls to below 2%. The salts also diminish in quantity while the sugar increases steadily towards the end of lactation. König gives the following analysis of human milk at the ninth month after parturition.

Proteid	Fat	Sugar	Ash
0.64	1.69	7.66	0.35

Accordingly we find that the growth during the first two months is most energetic. Camerer and Söldner have investigated the question of the daily increase of weight of children fed on mother's milk.

	3rd to 5th Week.	6th to 8th Week	9th to 10th Week	19th to 22nd Week.
Daily increase.	40 grams	30 grm	26 grm	19 grm.

The amount of the daily increase reaches in the 3rd to the 5th week its highest point at which time the average daily increase per kilogram of body weight is 1%. Along with a fall in proteid amount in the food the daily increase also falls so that the average daily increase during the first year is only .39%. Pröscher has found a corresponding condition in the case of the sheep.

2. A review of a series of analyses of milk reveals the fact that the milk of animals dwelling in warm or tropical climates is poor in heat-producing fat, while that of animals whose habitat is in colder or Arctic regions is richer in fat, even though the mother in such latitudes herself requires more fat for the maintenance of her body temperature. I subjoin a table of the same species before mentioned

in connection with the amount of proteids and of salts drawn up in the order of their richness in fats.

	Fat in 100 parts milk.
Mare	1.14
Ass	1.58
Camel	2.9
Cat	3.3
Man	3.48
Goat	4.1
Cow	4.5
Hippopotamus	4.5
Pig	6.88
Buffalo	8.25
Sheep	10.4
Dog	10.6
Reindeer	17.08
Elephant	20.58
Seal	43.76

It can easily be seen that in the first part of the list are animals inhabiting warm climates, while those animals which are subject to quite different temperature conditions are placed at the other end of the list. This is especially remarkable in the cases of the reindeer and the seal which have respectively 170 and 437 parts of fat in 1000 of milk, while the camel, the horse and the ass have as little as 29, 11 and 15 parts respectively in 1000 of milk. This corresponds to human experience that they who inhabit tropical regions prefer a diet wanting in fat, while a fatty food is preferred by the dwellers in the frigid zones of the North.

A remarkable exception is the case of the elephant. A teleological explanation for the anomalous position of that animal on the list is suggested. It inhabits only warm countries yet has a milk exceptionally rich in fat - 205 parts in 1000. Could we not form the hypothesis, not without support from other sides, that the elephant was originally an inhabitant of cold climates but has been driven to change its habitat to warm latitudes? The composition of its milk remains unchanged and tells the story of the past history of the race.

3. The third proposition stated was that animals which are compelled to early muscular exertion will receive milk adapted to supply the materials requisite for the production of muscular energy. In the first place it must be remembered that muscle can build up its proper structure only out of proteid, whereas it is still an open question which of the elementary foodstuffs act by their oxidation as the producers of muscular energy. It is to be expected that those animals which are fed by milk rich in proteid will be the first to build up a musclature sufficient for the needs of locomotion, and a glance at the list I have given of animals in the order of the richness of proteid in the milk of their species, u/

shows that man who is the latest of all to lead an active independent existence has the poorest milk so far as proteids are concerned, while such animals as the sheep and the cat which a few days after birth show considerable muscular activity are fed with milk containing an abundance of proteid. The famous experiments of Fick and Wislicenus showed that during a period of severe muscular exertion more work was done than could be accounted for by the oxidation of proteid. Similar results have been obtained by later investigators, and the tendency is to believe that non-proteids are the main producers of muscular energy. The two animals I have mentioned together with the horse, ass and elephant have milk rich in carbohydrate while man during the earlier part of lactation has milk poor in sugar. But as the child grows and gains in efforts towards muscular exertion, the milk with which it is nourished, becomes richer in milk sugar. In the selection of this constituent also the mammary gland seems to adapt itself to the needs of the growing child. The following figures from König bear witness to this:

Woman's milk 4 days after parturition has 4.11 parts Milksugar per cent.

Woman's milk 1 month after parturition has 6.18 parts Milksugar per cent.

Woman's milk 9 months after parturition has 7.66 parts Milksugar per cent.

The foregoing facts serve to illustrate the selective power of the mammary gland. The cells of the mammary glands of different animals select from the blood-plasma of the mother those constituents which are adapted to meet the requirements of the suckling for whose maintenance the milk is provided, but it is more remarkable that the gland of an individual animal should change the proportions of the constituents it selects to meet the changing conditions of the growing suckling. The exercise of the power of selection is at all times a wonderful phenomenon but that power exemplified in the secreting cells of the mammary gland is all the more remarkable since the material selected is for the support of a distinct organism. Between the food taken by the mother and the tissues of the child there is a long road to travel and many and Protean are the changes which the food constituents have to undergo before they become integral part of the tissues of the child. The bodily functions of the mother during the period of lactation become subservient to the function of lactation. It throws a great burden on all her tissues and how great is the burden may be proved by the fact that mental derangement, - lactational insanity - may résult from the constant drain put upon the system of the mother. In order to further investigate this important biological problem I set before me the task of making chemical examination of the tissue of the mammary gland and of examining from some points of view the composition of milk, in order to institute a comparison between the material from which the constituents of milk are selected, the selected material and the selecting tissue itself.

Very little work has been done on the chemistry of the mammary gland. Textbooks of physiology are almost silent on the subject and the only literature I have been able to obtain bearing on the question is in a paper by Hammarsten in which he deals principally with the chemistry of the nucleoproteid of the pancreas, but in which he alludes to the nucleoproteid of the mammary gland and compares it with that which may be obtained from the pancreas. I obtained from the slaughter-house the milk of cows removed just before the slaughter of the animal and also the udder, and both of these I subjected to chemical examination. To complete the picture an examination of the blood-plasma would also have been necessary but this I was unable to undertake. I propose first to deal with the chemistry of the blood plasma, then with that of the gland, and lastly with that of the selected material.

The Blood Plasma.

Milk takes its genesis from the blood, but not or only in small part in the manner that it is simply drawn from the blood, but its constituents serve for the building up of the cells of the mammary gland, and these elaborate the fully formed milk. If milk were simply an exudate from the blood its composition would correspond with that of the blood-plasma but it does not, and both bloodplasma and milk show remarkable constancy in their composition. There is no caseinogen in the blood. There is no milk-sugar in the blood. And there are fats present in milk which are unknown in the blood. But it is in the salts that the most noteworthy difference between the two exists. Bunge, gives the following as the composition of bullock's blood serum.

	In 1000 grams.
Water	913.3
Proteids	73.2
Other Organic Substances.	5.6
Inorganic Matter	7.9

The ash is in the following proportions.

	In 7.9 parts of ash from 1000 parts blood serum.
K_2O	0.254
Na_2O	4.235
CaO	0.126
MgO	0.045
Fe_2O_3	0.011
Cl	3.717
P_2O_5	0.266

The organic constituents are nitrogenous and non-nitrogenous. These include, besides the proteids, carbohydrates and fats but they are, compared with the amounts in milk, very small. The principal carbohydrate is dextrose which in man does not exceed the small figure of .12%. The others which exist only in traces, are glycogen and a carbohydrate which may be converted by boiling into a non-fermentable, reducing substance and has been termed animal gum.

The fats are also small in amount - from .2 to .5 or 1% and are composed of the glycerides of fatty acids - palmitin, stearin and olein.

The non-proteid nitrogenous organic substances include urea, creatin, creatinin, xanthin and hypoxanthin, and these substances are also present in milk, but their presence is of no practical importance.

The proteids are serum-albumin, serum-globulin and fibrinogen and a nucleoproteid. The total percentage of proteid is greater than the percentage of proteid in milk, but the form in which they exist is quite different. The serum-albumin certainly resembles lactalbumin but this is only present to any considerable amount in milk during the early days of lactation when colostrum is being secreted. It is said to differ from serum-albumin in the temperature at which it coagulates, but this is an unimportant difference as it may be greatly affected by other conditions. The nucleoproteid in blood-plasma is unimportant and is entirely due to the presence of white corpuscles, and is not present in the upper layers of the plasma which separate out from the blood enclosed in an isolated vein.

The comparison between the salts of the blood-plasma and those of the milk is reserved till a later period.

<u>The Mammary gland and the antecedents of milk.</u>

The economy of the mother early begins to prepare not only for the development of the foetus in utero but also for its nourishment outside the body. The mammary gland begins to enlarge shortly after conception and long before the birth of the foetus takes on its own peculiar function. As a means of studying that function I examined the udders of cows in a state of active lactation and in a general healthy condition.

In the first place the glands were cleaned of fat and as far as possible of areolar tissue, and after being cut into pieces were washed with water to get rid of any fully formed milk which might remain in the ducts. Then the whole gland was weighed and a carefully weighed portion was dried in a drying oven at a temperature of 105° C to estimate the water percentage.

The average water percentage of the gland is 72.1, the remainder being organic matter, nitrogenous and non-nitrogenous and ash.
The total solids, therefore, amount to 27.9% of the whole.

Ash - A weighed portion was incinerated for the estimation of the total ash and the average percentage of the material I found to be 3.83.

Nitrogenous matter. - A carefully weighed portion of the dried substance was subjected to examination for the total Nitrogen and Phosphorus.

Method:- The method employed was the Kjeldahl - Weibull, a modification of the wellknown Kjeldahl method, which enable one readily to estimate the total amount of Phosphorus as well as that of Nitrogen. A portion of organic matter of known weight is incinerated in a flask with pure Sulphuric acid, one or two crystals of Cupric Sulphate and ten or twelve grams of Potassium Sulphate. I found it advantageous to incinerate firstly for a time without the potassium sulphate, and after a time to allow the flask to cool and then add that salt. In this way the process is hastened and the risk of loss by bubbling is diminished. To the clear greenish fluid which remains distilled water is added making it up, when cool, to a certain volume in a standardised flask. This is divided into two known quantities and one is treated for nitrogen the other for phosphorus estimation. The former is made alkaline with strong solution of Sodium Hydrate and Ammonia is set free which is distilled over into a measured quantity of fifth-normal solution of oxalic acid. The oxalic acid remaining uncombined is titrated against a fifth-normal solution of sodium hydrate, phenol-phthalein being used as the indicator. The amount of oxalic acid combining with the ammonia is thus known, and, multiplying by the factor 2.8, the result is the amount of Nitrogen in milligrams.

The other portion is made alkaline with ammonia, and again acidified with nitric acid and a solution of ammonium molybdate in nitric acid added. This is kept for some hours at a temperature of 40° or 50°C and a precipitate of Phospho-molybdate of ammonium separates out. This precipitate is collected on a filter paper, and the filtrate tested by the addition of more Ammonium molybdate to find if any phosphorus remains unprecipitated. The precipitate is washed with dilute nitric acid solution in which it is quite insoluble, and dissolved in a 1 in 3 solution of Ammonia. To this is added a quantity of a magnesia mixture made of magnesium chloride, Ammonium chloride and ammonia. A precipitate of magnesium phosphate is formed which is collected on a filter paper, and incinerated. The ash is magnesium pyrophosphate, $Mg_2P_2O_7$, and from this the amount of Phosphorus is easily calculated.

The total amount of nitrogen present in 100 parts of fresh gland substance I found to be 1.311 parts. If this be all estimated as proteid Nitrogen - the non-proteid nitrogenous bodies being for the present disregarded, then one finds, using the factor 6.5, that the percentage of proteid in the mammary gland is 8.52.

Fat. - For the estimation of fat I used a method similar to that recommended by Stoklasa for the fats of milk.

A carefully weighed portion of the fresh gland, minced, is taken and mixed with a quantity of pure sand, which has been previously washed with alcohol and ether. This mixture was then dried first over a water-bath and then in an oven at 80°C. The sand prevents any loss of melted fat. Then the mixture is placed in a filter paper of suitable "cartridge" shape, and extracted with hot ether in Soxhlet's extraction apparatus for 30 hours. The ether containing the fat in solution is partly distilled off and the rest poured into a vessel such as a piknometer of known weight. The remainder of the ether is then evaporated in a water bath at 80°C, and in a vacuum chamber over Sulphuric Acid.

The weight of the fat thus obtained showed the high average of 10.06 parts in 100 of fresh mammary gland.

The remainder of the solid substance of the gland - the non-nitrogenous, and non-fatty material, may be considered to be carbohydrate, and this gives the analysis of mammary gland substance as follows:-

In 100 parts fresh gland.

Water	72.1		
Total Solids	27.9		
		Nitrogenous matter estimated as proteid	8.52
		Fats	10.06
		Carbohydrate	5.49
		Ash	3.83

The proteid of the mammary gland cells.

Hammarsten, in a paper on nucleoproteids, on which much work has been done during the last few years, was led to a consideration of proteid substances from which sugar might be split off in cases of diabetes. There are certain cases of glycosuria in which the origin of the sugar in the urine cannot be traced to glycogen or other carbohydrate in the body and in those cases it seemed that it might be produced in the decomposition of proteid. In searching for such a proteid he found that without difficulty there could be obtained from the pancreas, the liver and the mammary gland, proteid substances from which on boiling with a dilute acid, a reducing body could be separated. He did not, however, prosecute research into those of the liver and the mammary gland, but only into the pancreas proteid. I have separated and examined such a body obtained from the cow's udder.

The finely minced, clean, quite fresh mammary gland substance was boiled for twenty minutes in a very dilute solution of sodium carbonate - 5 pro mille. An opaque, light yellow, creamy-looking filtrate was obtained. On this being allowed to cool the fat or at least the greater part of it came to the surface and was removed in a firm yellow cake. Then to the filtrate acetic acid was added in sufficient amount to give the mixture an acid strength of 5 or 10 pro mille; and a very abundant, white, flocculent precipitate, closely resembling the precipitate of caseinogen which can be obtained from milk by the same method, separated out. This was allowed to fall and the superlatent clear fluid was siphoned off and kept. The precipitate, removal in this way was a Nucleoproteid.

It was purified by dissolving in dilute solution of sodium carbonate in which it freely dissolves, and again precipitating by acetic acid. This was done several times in order to ensure, as far as possible, thorough purification from inorganic salts.

The solution of this substance in an alkali gives no reaction to Trommer's or Fehling's test.

The precipitate was collected on a vacuum filter, and weighed. The total amount of the nucleoproteid obtained from a gland weighing 3,790 grams was 38.187 grams. In another case from a gland whose weight was 3,200 grams, nucleoproteid tonthe amount of 37.454 grams was separated, so that roughly speaking rather over 1% of the fresh gland substance or 3.5% of the total solids of the gland was nucleoproteid.

In the first case a portion of the dried proteid substance weighing 1.692 grams yielded 131.6 mgrms Nitrogen and 31.3 mgrms Phosphorus and in the other cases the results were very similar. The proportion of Phosphorus to Nitrogen varies but it does so also in pure caseinogen. This gives a percentage of 7.76 Nitrogen and 1.9% Phosphorus. These percentages are less than those of Nitrogen and Phosphorus in caseinogen so assuming that this nucleoproteid is the mother substance of caseinogen there must be present in combination with it some non-nitrogenous substance.

A part of the dried nucleoproteid was taken and boiled in a paraffin bath for 4 hours with a quantity of 2% Solution of Sulphuric acid, and the resulting solution showed very abundant reducing reactions with Fehling's and with Trommer's tests proving that by this means a carbohydrate can be split off from the nucleoproteid of the mammary gland. The amount of carbohydrate thus obtained was not estimated.

This sulphuric acid fluid also contains a certain amount of Nuclein Bases.

After boiling the nucleoproteid in sulphuric acid the solution was made alkaline with strong solution of barium hydrate. A very plentiful precipitate of Barium sulphate came down. This was filtered and a stream of carbon dioxide gas was passed through the filtrate in order to remove any excess of Barium hydrate which might be present. The precipitate of barium carbonate which appeared was removed by filtration and the clear filtrate made ammoniacal. By this means any Uric Acid or Guanin which is present comes down as a precipitate. The precipitate was collected on a filter paper and dissolved in hydrochloric acid. Any uric acid which is present should now separate out but none was found. The solution containing the Guanin was again made ammoniacal and that substance again separated out and was collected on a Nitrogen-free filter paper. The nitrogen present in it was estimated by Kjeldahl's method.

Then to the original ammoniacal solution silver-nitrate was added and the silver compounds of the other nuclein bases - xanthin, hypoxanthin and adenin appeared as a cloud. These were collected and the nitrogen in them estimated. The bases were not individually separated from one another but were estimated, from the amount of nitrogen found, in terms of hypoxanthin ($C_5H_4N_4O_3$)

The nuclein bases from 3.894 grams of the dried nucleoproteid amounted in terms of hypoxanthin to only 26.28 milligrams i.e. .675 parts in 100 of the nucleo-proteid (reckoned as hypoxanthin)

The remainder of the nucleoproteid amounting to 33.061 grams was then digested for 40 hours with .25% hydrochloric acid and liquor pepticus (Benger) at a temperature of 40°C in order to separate the pure nuclein. A clear fluid and a precipitate separated out. This precipitate, which separates from a nucleoproteid, is a body of the nuclein type either a true or a pseudo-nuclein.

No further splitting of this substance, such as would take place in tryptic digestion, into nucleic acid and a proteid, is caused by gastric digestion although the nuclein slowly dissolves. That this is the case was proved by Milroy's test. The fluid of the digested substance showed no precipitation when syntonin was added to it, a result which takes place in the presence of free nucleic acid, an artificial nuclein being formed. The clear filtrate of the digested substance gave a beautiful pink buiret reaction, due to the presence of peptone, arising from proteolysis of the proteid part of the nucleoproteid. It also reacted to Fehling's and Trommer's tests, showing that a carbohydrate reducing substance is split off from the nucleoproteid of the mammary gland during gastric digestion and hydrated in the presence of the hydrochloric acid.

The pure nuclein or paranuclein precipitate obtained from the 33.061 grams of nucleoproteid digested amounted to 1.627 grams so that the nucleoproteid which can be extracted from the mammary gland has the percentage of 4.7 of nuclein or paranuclein.

The precipitate, when examined by the Kjeldahl Weibull method showed 13.034% of Nitrogen and 5.346% of phosphorus. The high percentage of phosphorus present agrees with the results of Kossel who finds from 4 to 6% of phosphorus in nucleins, and disagrees with the results of Halliburton who states that he has never found in the nucleins he has prepared from various organs a higher percentage than 1%.

From the facts I have ascertained regarding the nucleo-proteid of the mammary gland I conclude that it is a highly organised molecule consisting of

$$\begin{cases}\text{a proteid radicle}\\ \text{a nuclein radicle}\\ \text{a carbohydrate radicle}\end{cases}$$

I shall leave over the discussion of this substance as an antecedent of some of the constituents of milk in the meantime.

I do not propose to enter into the nature of the fats of the mammary gland. I have already given, in my table of analysis of the gland substance, the percentage of fat which was found; but, although the gland was cleaned so far as was possible by dissection from fat in the connective tissue of the gland, yet undoubtedly the high percentage is in part due to fats not inside the cells but between the acini.

Nor do I intend, at this stage, to speak of the carbohydrate of the gland substance. Suffice it to say that in the fluid extract of the gland substance from which I removed the nucleoproteid, a very abundant reduction was obtained with Trommer's and Fehling's tests.

The Salts of the Mammary Gland.

The percentage of ash in the mammary gland is 3.83. I incinerated weighed portions of the gland substance, and estimated the amounts of the more important elements present, namely, Calcium, Iron, Sodium, Potassium and Phosphorus.

Methods:-

Calcium:- The ash was extracted with dilute hydrochloric acid, and the filtered fluid made ammoniacal. Then it was made acid with acetic acid and a solution of ammonium oxalate added. A precipitate of Calcium Oxolate appeared and this was collected on a filter paper, dried and incinerated in a platinum capsule over a blow-pipe. The calcium oxalate on the application of sufficient heat becomes converted into calcium carbonate and on further heating into calcium oxide. This ash was weighed and from it the amount of calcium present in the gland tissue can easily be estimated.

Iron : A weighed portion of gland substance dried to constant weight was incinerated with the addition of some sodium carbonate to fix the iron, and the resulting ash extracted with hot water. The insoluble residue was collected on an ash-free filter paper and washed with hot water. Now the filter paper containing the insoluble residue was put in a platinum capsule and incinerated till all carbon had disappeared. This ash was now extracted with hydrochloric acid and treated with the addition of Ammonium acetate. A precipitate of ferric phosphate now fell out, was collected on a filter paper, dried incinerated and weighed. From this the amount of Iron can easily be calculated.

Sodium and Potassium: The ash of a weighed portion of gland substance was extracted with hot water. To the filtrate Barium chloride was added as long as a precipitate appeared and then Barium hydrate until the mixture w was strongly alkaline. Then this was filtered and the precipitate washed with water, the washings being added to the filtrate, to which ammonia and ammonium carbonate were now added. Then the collected filtrate was evaporated to dryness and incinerated until it glowed feebly to get rid of ammonia. (This may be again dissolved, filtered, evaporated and incinerated to ensure purity.)

The resulting substance is a mixture of potassium and sodium chlorides.

These salts were now dissolved in a little water to which alcohol had been added, and then solution of platinic chloride in alcohol was added until no further precipitation appeared. After standing for twelve hours the precipitate was collected on a small weighed filter paper, washed with dilute spirit and dried at a temperature of 105°C. The precipitate is potassium platinic chloride and from the weight of this one can reckon for potassium chloride and, subtracting this result from the weight of the mixture of sodium and potassium chloride, obtain the weight of the sodium salt. By this means the amounts of sodium and potassium in a given amount of gland tissue can be easily estimated.

Phosphorus: The method of estimating the total phosphorus in a portion of organic substance has already been described.

In 100 grams fresh mammary gland there were found

Potassium	.09 grm
Sodium	.39 "
Iron	.033 "
Phosphorus	.802 "
Calcium	1.43 "

Reckoned in a different fashion, and in the manner in which the salts of milk are elsewhere given, we find that the ash of the mammary gland is approximately, since magnesium and chlorine were not estimated as follows:-

	In 100 parts ash:-
CaO	28.0
K_2O	3.01
Na_2O	14.9
Fe_2O_3	1.3
P_2O_5	52.7

The large amount of phosphoric acid is striking, but it is accounted for by its presence in the nucleoproteid of the cell protoplasm, in the nuclei of the cells, in inorganic salts, and in the substance now to be described

Phosphocarnic Acid in the Mammary gland and in milk.

Another phosphorised constituent of the mammary gland which I have isolated is Phosphocarnic acid. This substance has been recently described by Siegfried as a constituent of muscle and of milk, and it is undoubtedly of great importance. The method of separating it from the mammary gland was as follows:-

> The fluid in which the gland substance was boiled for the separation of the nucleoproteid was kept after the nucleoproteid was removed and the inorganic phosphates were precipitated by the addition of Calcium Chloride and Ammonia. These were removed by filtration and the clear ammoniacal filtrate was rendered neutral by the addition of hydrochloric acid. Then this fluid was brought to boiling point and a 1% solution of ferric chloride added. A light brown flocculent precipitate now appeared and the addition of ferric chloride was continued until free ferric chloride showed itself - sulphocyanide of potassium being used as the indicator. The brown precipitate is the Iron salt of phosphocarnic acid and has been termed by Siegfried carniferrin. The amount of phosphocarnic acid is estimated by taking a portion of the carniferrin of known weight, incinerating it in the Kjeldahl method and estimating the amount of Nitrogen present. On multiplying the amount of nitrogen by Balke's factor 6.1237, the result is the amount of phosphocarnic acid.

From one gland weighing 3790 grams 4.74 grams of Phosphocarnic Acid were obtained, and from another weighing 3000 grams 3.645 grams of this acid were separated. It seems therefore to be a substance of very considerable importance, and it will be well at this point to take up the description of that substance as it is found in milk.

Carnic acid or Fleischsäure is the name given by Siegfried to a substance which he discovered in muscle and in milk. Its formula is $C_{10}H_{15}N_3O_5$ and it therefore is closely related to antipeptone, a product of digestive proteolysis described by Kühne. As usually described antipeptone is stated to have some sulphur in its constitution; but Fränkel has stated that if pure it is sulphurfree and is identical with carnic acid. In connection with this discovery Halliburton thinks that all our views regarding the products of digestive proteolysis require revision and that it will form an important clue to the problem of proteid constitution. Both in muscle and in milk carnic acid is united with phosphorus to form phosphorfleischsäure or phosphocarnic acid.

The method of its separation in milk is as follows.

> The caseinogen is removed by Hammarsten's method of precipitation by acetic acid. The lactalbumin is coagulated by boiling and removed by filtration. Then the filtrate is made alkaline with the addition of ammonia, and by adding some solution of calcium chloride the inorganic phosphates are precipitated and are removed by filtration. The ammoniacal solution is treated as described above for the preparation of the iron salt of the phosphocarnic acid of the mammary gland, and the resulting precipitate is washed several times with distilled water and then with alcohol and ether.

Carniferrin, the iron salt thus obtained, is a light brown powder. It is insoluble in water but freely soluble in alkalies and alkaline carbonates. It is separable not only with ferric chloride, in excess of which the already prepared carniferrin is redissolved, but also with other iron salts. Balke and Ide who revised Siegfried's work, and established its correctness, investigated its composition and have found that the amount of Fleischsäure may be reckoned by multiplying the amount of nitrogen found in carniferrin by the factor 6.1237. The method I have employed in my experiments on phosphocarnic acid has in most cases been, not to weigh the carniferrin at all, but simply to incinerate it and estimate the amounts of nitrogen and phosphorus in the Kjeldahl-Weibull method already described. Pure carniferrin contains 35% Iron, 6% nitrogen and 1% Phosphorus.

That the iron precipitate is really a pure salt of phosphocarnic acid and not a mixture of a quantity of iron compound of carnic acid with a quantity of iron phosphate, is shown by the facts, that the iron compound is precipitated out of an alkaline solution from which all phosphates have been removed, that the precipitate obtained by ferric chloride from solutions of pure carnic acid is not soluble in alkalies, and contains over 50% Iron whereas the phosphocarnate of iron contains only 35%, and that by the addition of baryta water to the iron precipitate at ordinary temperature the barium salt of phosphocarnic acid is formed.

If one add to a solution of carniferrin in an alkali some acetic acid and a drop of ferrocyanide of potassium no Berlin-blue reaction appears at once. The reaction may come slowly by boiling, but quicker on the addition of mineral acids. This shows the firm nature of the combination with iron. It is easily split by strong hydrochloric acid at ordinary temperatures, and with the iron thus set free a typical red reaction with sulphocyanide of potassium appears.

Now it strikes one at once that this organic iron salt, soluble as it is in dilute alkalies, may come into a state of solution when acted on by the alkaline pancreatic juice if taken into the intestine and may be valuable as a soluble and absorbable organic iron salt. A series of experiments on the absorption of carniferrin have been described by Hall. His paper is valuable in that it gives a digest and criticism of all the historic work that has been done on the relative absorbability of organic and inorganic salts of iron. Clinical observation points to the fact that inorganic iron compounds are of benefit in cases of anaemia in producing an increase of haemoglobin. All iron in the body exists in organic combination, and if inorganic iron salts are absorbed they must unite immediately with proteid bodies to form organic compounds. Carniferrin is an iron compound of a proteid body. Bunge has stated that the benefit derived from giving inorganic iron salts is due rather to a saving of the organic iron compounds of the food, and that these alone are absorbed, and of benefit in the formation of haemoglobin.

Carniferrin has certain peculiarities in common with Bunge's haematogen, Schmiedeberg's ferratin and other organic compounds of iron.

1. It is soluble in dilute alkalies.
2. It gives no immediate reaction with ammonium sulphide.
3. It gives the Berlin-blue reaction slowly in the cold, quicker on boiling, and still quicker on the addition of mineral acids.

Hall gave carniferrin to half-grown mice and came to the conclusion that it is readily absorbed, the animals fed with it increasing their haemoglobin amount quicker than animals of the same birth to which no additional iron was given in the food, that it is absorbed by the bloodstream, and that it is quite innocuous.

It is therefore possible that phosphocarnic acid taken in the food may be of value in forming soluble organic salts of iron with any iron which might be taken, thus rendering the iron readily absorbable.

Not only does phosphocarnic acid form a soluble salt with iron, but it also forms alkali soluble salts with calcium, magnesium, barium and other metals.

Another peculiarity of carnic acid is that it combines with hydrochloric acid, not to form a salt but a conjoint acid. "The fact", says Siegfried, "that in animal bodies an acid is present which unites freely with hydrochloric acid is very remarkable. With the help of carnic acid, hydrochloric acid can be carried in the juices of the body, and the compound does not possess the peculiarities of free hydrochloric acid or of its salts, while the hydrochloric acid is separable from it by means of water".

These facts are sufficient to show the great importance of this substance, which is an almost constant constituent of milk.

Wittmaack has investigated the amounts of Phosphocarnic acid in the milk of cows, goats and the human female. He finds that the amounts are very constant and are as follows:-

In	cow's	milk	.056%
"	goat's	"	.110%
"	woman's	"	.124%

Thus in woman's milk there is more than double the quantity of Phosphocarnic acid that there is in cow's milk, and that in goat's milk is almost double the amount in cow's.

I have found, examining the milk of cows late in lactation - fully a year after calving, - and colostrum milk - the first milking after calving, - that there is not the constancy which Wittmaack states to be present. I found in the milk of a cow thirteen months after calving the relatively large amount of .1126% phosphocarnic acid, and in colostrum milk in one case .012%, in another .010%, and in a third, so little that it could not be estimated.

In the difference between cow's milk and human milk as regards phosphocarnic acid or Nucleon, as Siegfried suggests it should be called, a new distinction presents itself, and this difference is of especial significance considering the nature of Nucleon. It is very rich in phosphorus - the richest phosphorus holding body in milk. The total amount of phosphorus in cow's milk, reckoned as P_2O_5 is 1.5 grams %, in woman's .47 grams %; so that in cow's milk the phosphorus in phosphocarnic acid is 6% of the total phosphorus whereas in woman's the nucleon-phosphorus is 41.5% of the whole. Thus in woman's milk, taking into consideration the phosphorus in casein and in lecithin, the greater part of the phosphorus is organically bound; but in cow's milk more than half the phosphorus is in inorganic combination.

It seemed to me remarkable that such a considerable amount of this substance was present in the mammary gland of cows and so small an amount in milk and I determined to investigate the nature of the whey-proteid described by Hammarsten as split off from caseinogen during coagulation by rennin. He has stated that when casein is formed by the action of rennin the caseinogen is not simply converted from a soluble into an insoluble proteid, but the caseinogen molecule is split into two parts. One of these is said to become united with a calcium salt to form the curd or casein; the other is a soluble proteid and passes into the whey. This body has been termed the whey-proteid and some observers have stated it to be a proteose or peptone. It is not coagulated by heat and rennet has no further action on it.

I obtained a specimen of cow's milk, 200 C.C. in amount, and divided it into two equal parts. The one I diluted with water and to it I added acetic acid to precipitate the caseinogen. Then I removed the lactalbumin and the inorganic phosphates in the manner already described. The other half of the milk I heated to 40° C. and then added 20 C.C. essence of rennet (Mackenzie's). By this means a thick curd of casein appeared and on its being broken up the whey separated. The curd was subjected to pressure in a clean linen cloth and the whey thoroughly removed. Then the curd was washed with water to remove any whey which might remain in it, and the wash-water added to the whey. This was now filtered to get rid of any particles of casein which might have got through the linen cloth in which it was pressed, and the lactalbumin and inorganic phosphates removed.

Thus I had two fluids A and B, containing in the former case only the phosphocarnic acid of the milk some soluble salts such as chlorides and the milk-sugar, in the latter case these constituents and in addition the whey-proteid separated during the action of the rennin.

In these fluids the iron salts of phosphocarnic acid was prepared by Siegfried's method. The carniferrin was washed several times with distilled water to get rid of salts and sugar, and then with alcohol and ether to remove any of the fats of milk which might remain, and incinerated in the Kjeldahl-Weibull method for the estimation of nitrogen and phosphorus.

The carniferrin of A showed	24.5	milligrams	Nitrogen
	14.5	"	Phosphorus
The carniferrin of B showed	39.9	"	Nitrogen
	22.1	"	Phosphorus

The additional nitrogen and phosphorus in the latter case - that in which the casein was coagulated and rennin, - could not be due to foreign substance since the carniferrin was washed free from any impurities, and no iron precipitate is produced by treating "essence of rennet" with ferric chloride in the manner required for the precipitation of carniferrin.

It appears therefore that the substance split off from caseinogen by rennin, which has been described as a peptone, is really identical with phosphocarnic acid. This was corroborated by the examination of several specimens of milk in a similar way.

The probability, since the body appearing in the whey after treating milk with rennin is an organic phosphorus building body, is that caseinogen is the constituent of milk from which it is separated, but in order to prove this I performed the following experiment. A large quantity of milk was obtained and two portions of it amounting to 500 C.C. each were treated in the way described above, while from another 500 C.C. the caseinogen was removed and treated as described below.

A. 500 C.C. Caseinogen precipitated by acetic acid, lactalbumin and inorganic phosphates removed, and carniferrin prepared. The amount of phosphocarnic acid was estimated by ascertaining the amount of Nitrogen in the carniferrin and multiplying by Balke and Ide's factor, 6.1237, the phosphorus was also estimated.

B. 500 C.C. Casein curd made by 20 C.C. "essence of rennet". Whey separated, lactalbumin and inorganic phosphates removed and carniferrin prepared, and the phosphocarnic acid estimated in the same way.

In these two cases A showed 51.1 milligrams nitrogen in the carniferrin separated from it and this, on multiplying by Balke's coefficient, gave the result that the phosphocarnic acid in that specimen of milk amounted to .062%, a result which is almost identical with the percentage stated by Wittmaack to be the normal amount in cow's milk. The amount of phosphorus was 31 milligrams.

B showed in the carniferrin prepared from it the large amount of 122.30 milligrams nitrogen, and using Balke's factor this shows .161 grams of phosphocarnic acid in each 100 grams of milk treated with rennin.

In the third 500 C.C. of the milk the caseinogen was precipitated by acetic acid and was collected and washed several times with distilled water in order to remove the inorganic salts. Then this caseinogen was dissolved in a dilute solution of sodium carbonate, and this solution was divided into two equal parts each of which, therefore, contained the caseinogen of 250 C.C. milk.

One of these solutions (C) was made neutral with hydrochloric acid, heated to 40° Centigrade and 20 C.C. essence of rennet added. This was allowed to stand at this temperature for half an hour and no coagulation occurred since the caseinogen had been washed free from calcium salts. Then some dilute solution of calcium chloride was added and a coagulum of casein was immediately formed. This was allowed to stand for some time. Then the casein was removed and from the fluid filtrate the phosphocarnic acid was precipitated by ferric chloride in the usual way. The amount of phosphocarnic acid was estimated by Balke's method.

The other half D was also heated to 40° C. and 20 c.c. essence of rennet added. No coagulum appeared, and then the fluid was boiled to destroy the activity of the rennin. Then a solution of calcium chloride was added and, although the action of the rennin had been destroyed, a coagulum of casein appeared. In this case also the phosphocarnic acid was estimated.

In case C the carniferrin showed 56 mgrm N. and 13 mgrm P.

In case D the carniferrin showed 65.5 mgrm N. and 15 mgrm P.

Thus it appears that a nitrogenous and phosphorised substance forming an iron salt identical with the carniferrin of Siegfried, is split off from caseinogen by the action of rennin, that this action takes place independently of the presence of calcium salts, since in specimen D the action of the rennin was stopped by boiling before the calcium salt was added. This split off body, from its action with a ferric salt, appears to be phosphocarnic acid.

In another specimen of the same milk the amount of phosphocarnic acid split off from a weighed portion of caseinogen was estimated. The percentage of caseinogen in this milk was found to be 2.56.

Therefore in case D, in which example the caseinogen separable from 250 c.c. milk was employed, the total amount of caseinogen acted on by rennin was 6.4 grams. From this, phosphocarnic acid containing 65.5 mgrm nitrogen was split off, i.e. from the 2.56 grams of caseinogen in 100 c.c. milk 13.1 mgrm nitrogen were split off and entered into the carniferrin. Using Balke's factor, therefore, we find that .070 grms of phosphocarnic acid are separated by the action of rennet from 100 c.c. milk, or from 2.56 grams caseinogen. It is possible that a longer action of rennin may have greater effect in this direction.

It remains to enquire if any other body corresponding to the whey-proteid of Hammarsten is split off from caseinogen by the action of rennin.

> In order to ascertain this a specimen of 500 c.c. milk was obtained. The caseinogen was precipitated, by acetic acid and thoroughly washed with distilled water. The weight of the undried caseinogen was ascertained and a small portion of known weight was taken and dried to constant weight and then the nitrogen in it estimated by the Kjeldahl method. The total amount of nitrogen in the caseinogen was thus ascertained. The rest of the caseinogen was dissolved in dilute solution of sodium carbonate, and, after the solution had been made neutral by hydrochloric acid was acted on by 20 c.c. essence of rennet at a temperature of 40° C. Then the casein was separated by the addition of calcium chloride, weighed, and the total amount of nitrogen in it estimated in the manner above described. After removal of any inorganic phosphates which might remain the phospho-

> carnic acid was precipitated as carniferrin and the total amount of Nitrogen present in this form was ascertained. If phosphocarnic acid is the substance split off from caseinogen by the action of the rennin, then the sum of the nitrogen in casein and that in phosphocarnic acid, should be equal to the amount of nitrogen in the caseinogen.

The total amount of nitrogen present in the caseinogen was 1674.1 milligrams. Of this 91 milligrams were contained in the quantity employed for nitrogen estimation The total amount in casein was 1543 milligrams and in the carniferrin 59.5 milligrams.

Nitrogen in caseinogen	1674.1 mgrm	Nitrogen in casein	1543	mgrm
Nitrogen in part used for estimation.	91	Nitrogen in carniferrin	59.5	"
	1583.1		1602.5	

Allowing for slight errors which cannot fail to creep into such chemical calculations, the figures are sufficiently close to show that no nitrogenous body is split off from caseinogen by the action of rennin save phosphocarnic acid.

Therefore I conclude that the substance called by Hammarsten "Whey-proteid" is phosphocarnic acid, and is identical with the body described by Siegfried as already existent in milk. The action of rennin on caseinogen therefore seems to be

Caseinogen + Rennin. → Phosphocarnic acid
→ Soluble Casein + Calcium Salt. = Coagulated Casein

The formation of the constituents of milk.

I do not propose to enter fully into a description of the constituents of milk. They are sufficiently well known. But by way of comparison with the analysis already given of the constituents of the mammary gland let me give an average analysis of the milk removed from the animals whose glands were examined.

In 100 grams milk.

Water	85.35	
Total Solids	14.35	
	Nitrogenous substances	5.55
	Fats	3.45
	Carbohydrate	4.42
	Salts	0.87
	Phosphocarnic Acid	0.060

That the protoplasm of the mammary gland cells breaks up to form the secretion is beyond doubt. The cells which during the resting stage have been actively growing and elaborating the substances which are to form the secretion become higher, have frequently more nuclei and have particles apparently fatty in their outer part. Then this outer part breaks down, and milk with its caseinogen, its fatty globules, its sugar its salts and its large percentage of water is thrown into the ducts.

<u>The Proteids:</u> Undoubtedly serum albumin and serum globulin are the percursors of the proteids of milk, and undoubtedly also the nucleoproteid separable from the mammary gland cells is a more recent antecedent of caseinogen. If one were led by the hypothesis of Wilson and Matthews that the nucleus is the formative part of the cell, then one might suppose that the proteids of the blood entering the cell are carried to the nucleus, there combine with a phosphorus-holding body and are again thrown off into the protoplasm of the cell as nucleoalbumin. Such a theory would seem to have some support from the facts that the cells have several nuclei, that those nuclei nearer the lumen are larger, and less deeply staining than the nucleus nearest the attached end of the cell, that these nuclei may appear lobulated as if about to throw off parts of their substance into the protoplasm, and that these nuclei themselves are thrown off into the secretion.

But the nucleoproteid separable from the gland is a much more complex molecule than is caseinogen, and its nuclein radicle is of a different nature. The nuclein of this nucleoproteid is a true nuclein, in part at least, since nuclein bases are separable from it; whereas the nuclein of caseinogen is a pseudonuclein containing no nuclein bases. Therefore the process of elaboration is much more complex than such a convenient hypothesis as that just mentioned would suggest.

The older belief that caseinogen is produced by a ferment action on albumin is probably incorrect. Kemmerich was led to this belief by finding that by digesting colostrum milk at the temperature of the body caseinogen is built up at the expense of albumin He took human colostrum kept it for 24 hours at 38°C and found the following change.

	20 C.C. fresh Colostrum.	20 C.C. colostrum after digestion. at 38° C.
Casein	3.125%	4.240%
Albumin	4.350"	3.060"

He ascribed this to a ferment which passed into the milk and there continued its action if exposed to a suitable temperature condition. But no such ferment has been isolated. The colostrum milk is full of still living cells, not only colostrum corpuscles but also wandering cells, and it is much more probable that this increase of casein at the expense of albumin is a result of the vital activity of the living protoplasm.

Thierfelder states that by the digestion of mammary gland substance at body temperature a body like caseinogen and probably caseinogen appears. By the addition of serum albumin the amount of this body is increased, and he draws the conclusion that its production is due to a ferment. Even more than in Kemmerich's experiments it is apparent that the production of this body is due to the vital action of the mammary gland cells.

If, as is very probable, the nucleoproteid separable from the gland is the forerunner of the nucleoalbumin of the milk, the question comes to be what happens to the nuclein-bases or the molecule containing them? They do not enter the milk, but remain in the gland cells probably to become active agents in the formation of new mother-substance in the resting cells, In the cell the following might be supposed to take place. From the serum albumin and serum globulin, and from organic phosphorus-holding bodies in the blood, a very complex phosphorus-holding molecule is built up. This molecule contains, as we have seen, a proteid, a nuclein and a carbohydrate radicle, and is kept in solution in the cell sap by the salts present. When secretion begins this complex molecule breaks up. It furnishes caseinogen which contains neither the nuclein bases nor the caebohydrate radicle of the mother substance, and the caseinogen passes into the milk in a state of suspension.

I have shown, in an earlier part of this paper, that the mammary gland contains a considerable amount of an organic phosphorus-holding acid viz; phosphocarnic acid. A certain amount of this substance, as Siegfried has shown, and I have corroborated is found in the milk, but before it can be separated from the milk, the caseinogen is exposed to treatment with acetic acid, and some caseinogen may escape precipitation and be exposed to boiling. Also I have shown that this substance is separable from caseinogen by the action of rennin, and again by the action of tryptic digestion. What part it plays in the formation of caseinogen it is impossible to say, but it is remarkable that it is found in the mammary gland before caseinogen is fully prepared, and can again be obtained by the decomposition of caseinogen.

Milk-sugar.

Bert described the production of a copper-reducing substance from the cow's udder by digestion of the gland at body temperature, and supposed it to be a mother-substance of lactose into which it was converted by a ferment. Landwehr found a similar substance in the mammary glands of rabbits and held a similar view regarding its formation. Such a ferment has not been isolated, and no ferment with this action passes into the milk.

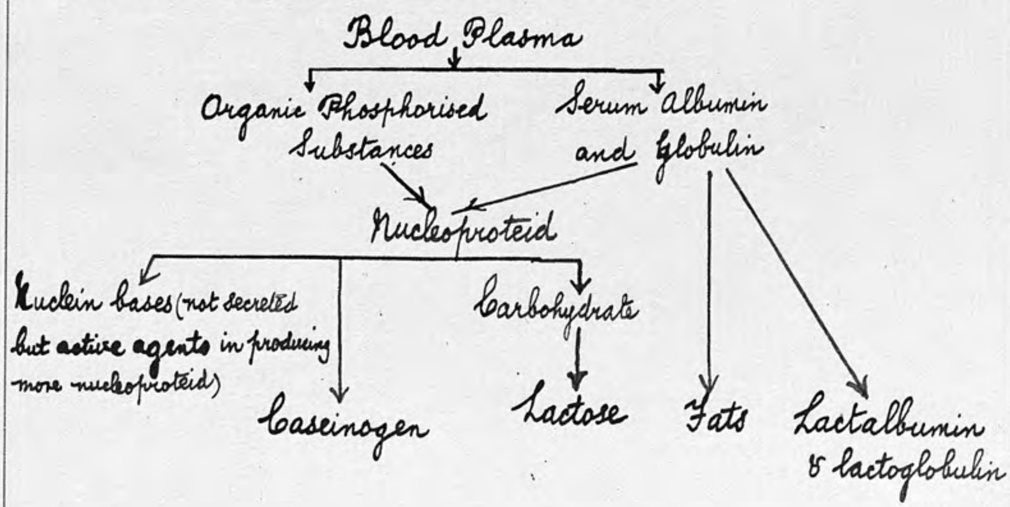
The facts regarding the ultimate source of milk-sugar are well known. Lactating animals fed on a purely proteid diet have plenty sugar in their milk, and the giving of increased amounts of carbohydrate in the food does not materially alter the amount of sugar in the milk. The percentage of sugars in normal blood is very small indeed, - in human blood only .12%, and there is no lactose in normal blood. Thierfelder has removed the mammary glands from goats and has stated that lactose appears in the urine after this operation. If this were really the case then it would argue strongly in favour of milksugar being preformed in the blood but there is no evidence of this from any other point of view, and it is doubtful, from what is now known of the wide extent to which the gland acini reach, if all the gland tissue was removed. In lactating women lactose sometimes appears in the urine but this is the result of absorption from the gland into the blood, and it is certain to occur if the breasts are not emptied of their secretion.

As I have shown the nucleoproteid of the mammary gland cells possesses a carbohydrate radicle, and it is probable that, when caseinogen is furnished from the complex mother substance, a soluble carbohydrate, differing in nature from the sugars present in the blood, is also produced. The complex nucleoproteid of the mammary gland is therefore, in all probability, the mother substance of lactose as well as of caseinogen.

Fats: Fats are present in blood only in a very small amount, but in the mammary gland the percentage is very high. In the gland cells during secretion little globules, stainable with osmic acid are visible in the superficial part of the cell, and during secretion these are thrown off to become the fat-globules of the milk. Although comparatively little is known of the interchangeability of organic substances, yet the known facts tend to show that the fats of the mammary gland are produced not from those of the blood, but from proteids.

There are fats in milk which are not present in the blood. The addition of fat to the food rather diminishes than increases the fats of milk. But a plentiful supply of proteid food increases the richness of the milk as regards fat, and the fat percentage of dog's milk when the animal was fed on purely proteid diet was found by Voit to be greater than when the animal was fed on a mixture of proteid and carbohydrate. The fat and the carbohydrate of the food seem to influence milk secretion only so far as they effect a saving of proteid substances throughout the body. The fats of milk, therefore, appear to arise from an alteration of proteid substances in the gland cells, and it is remarkable that alterations in the nature and quantity of the food affect the total amount of milk secreted but do not alter the proportions of the various constituents to one another.

A scheme of the formation of the constituents of milk may be drawn up as follows:-



Salts:

I have in an earlier part of this paper discussed the remarkable fact that the rapidity of growth of the suckling is in direct proportion to the amount of salts present in the milk. This is an illustration of the marvellous selective power of the mammary gland. I have previously dealt only with the salts in a collective way, but now shall take up the individual salts. Bunge estimated the amount of each individual salt in the whole body of a puppy, in dog's milk and in the bloodserum of the dog. He showed the remarkable fact that the salts in the body of the puppy are in the same proportion to one another as in the milk of the female dog, whereas the salts in the bloodserum of the dog are in quite different proportion.

	Body of Puppy In 100 parts ash.	Milk of bitch In 100 parts ash.	Dog's blood serum In 100 parts ash.
K_2O	8.5	10.7	2.4
Na_2O	8.2	6.1	52.1
CaO	35.8	34.4	2.1
MgO	1.6	1.5	0.5
Fe_2O_3	0.34	0.14	0.12
P_2O_5	39.8	37.5	5.9
Cl	7.3	12.4	47.6

The seat of selective activity is in the cells of the mammary gland in close relation to which there are lymph vessels and from the fluid in these vessels the gland cells [redacted] remove the constituents suited for the needs of the suckling and elaborate them into the milk.

I have examined the ash of the mammary glands of cows, and the relationship between the different constituents in that ash and those in the ash of the blood and the milk of the cow is interesting. I have not been able to obtain an analysis of the ash of blood serum of the cow, but for the purposes of comparison I give Bunge's analysis of the ash of bullock's blood, which cannot materially differ from that of cow's blood.

	Bullock's Serum In 100 parts ash.	Udder of Cow In 100 parts ash.	Milk of cow. In 100 parts ash.
K_2O	2.9	3.01	21.2
Na_2O	49.1	14.9	13.3
CaO	1.4	28.0	19.1
MgO	0.50	+	2.5
Fe_2O_3	0.13	1.3	0.04
P_2O_5	3.0	52.7	23.5
Cl	41.9	-	20.3

The analysis of the ash of the cow's udder is only approximately correct since the magnesium and the chlorine were not estimated.

In blood serum the chief acid is hydrochloric and the chief base sodium.

In the mammary gland the chief acid is phosphoric and the chief bases sodium and calcium, and in milk the chief acid also is phosphoric and potassium and calcium form the chief bases.

Sodium and Potassium: The relationship between these substances in lymph and in milk forms perhaps the best object lesson of the selective power of the mammary gland. Sodium chloride is the chief salt in lymph and if the salts of the milk were simply extracted from the lymph in which the gland cells are bathed it would be so also in milk. But what are the needs of the suckling? Before birth the child possesses cartilage instead of bone and its muscles are but poorly developed, and in order to fit it for an active extrauterine existence bone must replace cartilage and the muscles must rapidly develop. Cartilage is much richer in sodium than in potassium but in bone that relationship is reversed. In muscle also the amount of potassium exceeds that of sodium. Therefore as the suckling grows it becomes relatively richer in potassium and poorer in sodium, and thus the relative amounts of two substances in milk finds a **teleological explanation.** The mammary gland holds an intermediate position between the lymph on the one hand and the milk on the other as regards those substances. Its cells behave in the same manner as marine animals and plants, which, living in a medium very rich in sodium and poorer in other elements, yet take up comparatively little of the most plentiful substance and build up their structure on the less abundant salts. My analysis of the salts of the udder must have been influenced by the salts present in the connective tissue and in the lymph and blood remaining in the vessels of the organ, yet it sufficiently shows the intermediate position which it holds, as regards sodium and potassium, between the blood serum and the milk. Undoubtedly if it were possible to make an analysis of the secreting cells alone this intermediate position would be more pronounced.

<u>Calcium</u> is the only inorganic material we have carefully to provide for in the choice of an artificial child's food, and it is richly supplied in the food provided by nature for the growing child. Blood serum is poor in it, but the mammary gland extremely

rich, richer even than milk itself. It seems, then, that the gland cells, extracting it from the lymph, must store it up, and give it off to the milk in the quantities necessary. Calcium is present in nearly all foods, but is richest of all in milk with the single exception of yolk of egg; which, it must be remembered, is also a food selected to meet the needs of a growing organism.

The withholding of calcium from the food in growing animals causes rickets or a condition in which the bones have an appearance, macroscopically and microscopically indistinguishable from the appearance produced by that disease; and in adults want of lime in the food causes a condition known as osteoporosis. Baginsky made a series of investigations into the results of deprivation of calcium in the food of young dogs and at the same time into the effect of giving lactic acid in the food. Young dogs of the same litter were taken and put on a diet containing flesh, fat and water, the flesh having been well boiled so as to get rid, as far as possible, of calcium salts, although it is impossible to get proteid substances absolutely free of salts.

Dog 1 got this food without any addition.
Dog 2 got this food 2 grms calcium phosphate.
Dog 3 got this food 2 grams calcium phosphate and 2 grams Lactic Acid.

The animal which was given calcium salts grew and thrived, while the other two presented distinctly rachitic appearances, the bones being soft and thick and showing the typical microscopic appearances of rachitic bones. Chemical examination of the bones showed that the total quantity of ash was diminished but the individual constituents of the ash did not vary in their proportions to one another.

The experiments of Lunin, quoted by Bunge, are of interest in this connection. He fed animals on ash free casein, pure fat and pure cane sugar. They rapidly died. In other cases he added to this food inorganic salts in the proportions in which they are found in milk, and animals fed on this also died, while control animals fed on unchanged milk remained healthy and thrived. It appears from this that calcium, ~~as~~ and iron, can only be absorbed in organic combination. Practical experience has tended to show that water containing a large amount of calcium salts has not much influence in preventing the production of rickets. The giving of lime water to infants merely dilutes the milk so far as lime salts are concerned, since milk is richer in calcium than lime-water itself is.

In the bones calcium salts are organically bound, being deposited in the form of a proteid combined with phosphorus and calcium, and the condition of rachitis appears to be due to an absence of organic calcium salts in the food or to want of absorption of such salts.

I have previously spoken of the calcium salt of phosphocarnic acid which Siegfried was able to prepare. This organic acid may be of value to bind calcium and form an absorbable organic calcium salt. Siegfried has not been able to separate the salt quite free in order to estimate the amount of calcium it contains. But if it could bind calcium in equivalent amount to the amount of iron with which it combines to form carniferrin, then the quantity of phosphocarnic acid already existent in woman's milk will be able to take into combination more than all the calcium present in the milk. But the amount of phosphocarnic acid in cow's milk will not be able to take into combination more than a quarter of the calcium in cow's milk, and hence, says Siegfried, it is conformable with experience that cow's milk with its large amount of calcium and small amount of phosphocarnic acid, is less suitable for absorption and assimilation than human milk.

But Siegfried has not taken into account the fact that phosphocarnic acid is split off from caseinogen during the process of digestion, and thus cow's milk with its greater amount of proteid will probably allow more splitting off of this substance, and thus allow of the formation of a greater amount of the soluble and assimilable Calcium salt. It is a matter of everyday belief that a child thrives better on its mother's milk than on cow's milk, and that the feeble digestive powers of the young child can digest more easily the mother's than the cow's milk. That the digestive powers are feeble is demonstrated by the fact that a good deal of casein passes undigested in the dejecta and this is more true in the case of children fed on cow's than in those fed on mother's milk. Not only is cow's milk much poorer in already formed phosphocarnic acid but it is always, when given to young children, diluted with at least equal parts of water, so that in a given quantity of food, the child gets only a quarter or a fifth of the phosphocarnic acid which he would get if he were fed on his mother's milk; and in addition gets not only less caseinogen, but a form of caseinogen, from which his feeble digestive powers are less able to split off sufficient nucleon for the formation of a sufficient quantity of soluble organic calcium salt.

Probably rachitis never occurs without an accompanying derangement of the alimentary system. It occurs in infants subjected to improper hygienic conditions the most important of which is injudicious feeding. Its onset is accompanied by indigestion and diarrhoea. It is not improbable that this abnormal alimentary condition may prevent the formation or the absorption of such a calcium salt as that formed by combination of calcium and phosphocarnic acid. It is also worthy of mention that lactic acid which experimentally is capable of producing a condition very similar to rachitis is one of the decomposition products of phosphocarnic acid.

<u>Iron</u>: The quantity of iron in milk is at first sight astonishingly small. Bunge draws attention to the discrepancy in regard to iron in the similarity of the ash of a newborn animal and in the ash of its milk. He found the proportion of iron in the ash of milk to be much less than in the body of a newborn animal.

Fe_2O_3 in ash of newborn puppy 0.72%
" " " " dog's milk 0.12%

He finds an explanation in the fact that the young animallays up in its tissues a store of iron more than sufficient for its needs, and that with the increasing age of the animal the amount of iron per kilogram of body weight diminishes.

Puppy, per kilogram of body weight

Newly born	182	mgr Fe
10 hours old ...	112	
3 days old	96	" "
4 days old	75	" "
24 days old	32	" "

Winternitz, in a paper to which I have already referred, states that the amount of haemoglobin is much greater in newborn than in older animals. Zalesky has found that the liver of young animals seems to store up iron the liver of a newborn puppy having 4 to 9 times as much iron as that of an adult dog. The number of red corpuscles is also higher in the blood of the foetus than in that of the adult, and a greater amount of haemoglobin seems necessary for oxygenation during intrauterine than during extrauterine life, since the tension of oxygen in the blood on the maternal side of the placenta is comparatively low. Therefore when the infant is born it has more haemoglobin than it has need for, and the condition does not appear to be a storing-up of iron, as Bunge expresses it, but a result of the different physiological condition in which the child has been living before birth. It is the custom of some obstetricians to allow the child to remain attached to the placenta for some little time after birth in order that it may get as much of the blood in the placenta as possible into its body, and in some cases a jaundiced appearance results a few days after birth. This is due to a breaking down of the excess of haemoglobin, and I have no doubt that the huge amount of iron which Zalesky found in the liver is due to a breaking down of red corpuscles throughout the area of the portal circulation, and that the excess of iron is excreted in inorganic form. The very rapid diminution of the amount of iron in each kilogram of the growing body is in support of this view.

Bunge in support of his view that the body of the foetus stores up iron, begs the question of the difficulty of absorbing and assimilating compounds of iron. He says there are two roads by which iron can enter - the placenta and the mammary gland - and the former is the one chosen because the ironholding constituents of the milk might become in the intestinal canal of the child the prey of organisms and their absorption thus be prevented. Such foresight would be most astonishing.

But although the amount of iron in milk is remarkably small it is quite sufficient for the needs of the infant. Stockman has shown that in the ordinary food of a healthy adult only 10 milligrams of iron are ingested per day. Now an infant weighing 7 kilograms and taking one litre of human milk daily takes 4 milligrams, or .6 mgrm per kilo of body weight. The adult of 70 kilos takes 10 milligrams or only .14 mgrm per kilo of body weight. Surely there is sufficient margin between those figures to allow of increase of haemoglobin with increasing body weight.

I have already referred to the iron salt of phosphocarnic acid and it is not at all improbable that the iron of the food is absorbed in an organic salt of the same nature, if not indeed in that very form.

The high percentage of iron in my analysis of the mammary gland is probably due to the presence of a certain amount of blood in the vessels of the gland.

Chlorides as has already been pointed out do not take the important place in the food of the suckling that they do in that of the adult. Chlorides bulk very largely in the salts of the blood serum yet do not pass in any considerable amount into the milk. In the economy of the suckling the chlorides are not of much importance for building up tissues, but they enter into various secretions, especially the digestive fluids. They also seem to be of importance in aiding the elimination of nitrogenous substances, as is shown by the fact that diuretics increase the excretion of chlorides along with the increase of the excretion of nitrogenous matter.

Phosphoric acid is the chief acid of milk. The importance of phosphorus in growing tissues has been already emphasised and milk from its richness in organically bound phosphorus is adapted for the needs of the rapidly growing tissues of the suckling. In cow's milk the greater part of the phosphorus is in inorganic, in woman's in organic combination. The amount present in the phosphocarnic acid of these two varieties of milk has been given above.

I have examined in several specimens of milk the mode of combination of all the phosphorus present.

In a specimen of colostrum milk I estimated the total phosphorus in the milk, by incinerating a weighed portion of it and employing the ammonium molybdate method , and then separating the various phosphorus holding constituents and estimating the amounts present in them.

The caseinogen of a portion of milk of known weight was separated by Hammarsten's method, weighed, and a weighed part of it incinerated and the phosphorus estimated by the ammonium molybdate method.

Then the lactalbumin was removed and the inorganic phosphates precipitated by ammonia and calcium chloride. These were collected on a filter paper and dissolved in acetic acid. The acetic acid solution was measured and the phosphorus in a measured quantity of it estimated.

Then the phosphocarnic acid was prepared as carniferrin and the amount of phosphorus in this form determined.

Lastly the phosphorus present in lecithin was estimated. A weighed portion of the milk was taken, dried in sand after Stoklasa 's method and the fats extracted with warm ether in a Soxhlet's extracting apparatus. The fats were now saponified by mixing them with a solution of 3 or 4 grains potassium hydrate in water and 50 c.c. alcohol, and boiling in a flask over a water bath. The lecithin is also thus saponified. Then the fatty acids were separated from the soaps by pouring these into a hot 4% solution of sulphuric acid. The insoluble fatty acids separated out and the phosphoric acid of the lecithin, remaining in the solution, was estimated in the usual way. All the lecithin may not be separated in this way from the mixture of sand and milk so that this mixture was extracted with hot alcohol, and the alcohol evaporated. The residue from this was incinerated with the addition of some sodium carbonate and potassium nitrate, and the phosphorus found in this added to that separated from the fats.

Total P. in 100 c.c. Colostrum Milk.	P. in caseinogen of 100 cc.		
221.85 milligrams.	Colostrum milk	99.86	mgrms
	P. of phosphocarnic acid.	2.01	"
	P. of Lecithin	6.91	"
	Inorganic P.	104.95	"
		213.73	"

The difference may be partly accounted for by the phosphorus of glycero-phosphoric acid. In milk at a later period of lactation there is less inorganic phosphorus and more organic in caseinogen and in phosphocarnic acid than in colostrum.

Caseinogen has not a constant phosphorus percentage but varies within fairly wide limits. Bokai stated that the phosphorus in nucleins is not absorbed. He believed that the nucleins were not digested by tryptic digestion, and Baginsky, whose work was done from the point of view of child feeding, followed his teaching and fell into the singularly erroneous belief that all organically bound phosphorus appeared in the dejecta whereas the inorganic phosphorus alone was absorbed. Probably the exact converse is more correct. Sandmeyer and Gumlich have proved that both nuclein and paranuclein are digestible and absorbable and now Siegfried has pointed out the great importance of phosphocarnic acid. The probability is that milk is valuable as a food for the growth of the tissues of the suckling in proportion to the amount of organically bound phosphorus.

In this paper I have tried to show that the mammary glands of various animals select a food suited for the needs of the young of those animals. The practical bearing of this is apparent. It is an undoubted fact that human milk is the best adapted for the human suckling, and no food, milk of other animals or artificially prepared food, can with advantage compensate for the loss of the natural mother's milk. The milk most commonly employed to replace the mother's milk is that of the cow and the common practice is, after sterilisation, to dilute it with at least an equal quantity of water and to add sugar. Thus the caseinogen in a given quantity of milk is diminished in the mixture by at least a half. Langgard has shown that the caseinogen of human milk is more finely subdivided and more easily digested than that of cow's and thus the passage of clots of undigested casein is more common in children fed with cow's milk. I have already stated that cow's milk contains only one half as much phosphocarnic acid already present in the milk as human, and when the milk is diluted this amount is diminished to a quarter or a fifth. In this way a substance of great service in the absorption of salts in organic form is very considerably decreased in amount. As regards salts human milk contains them in the exact proportions required for the body of the suckling. In cow's milk the phosphorus is more than half inorganically bound: in human almost entirely in organic combination. If a sufficient quantity of diluted cow's milk is to be taken for the nutrition of the child, then more water and more salts are ingested than if the child were fed on human milk, and a greater strain is put not only on the digestive but also on the excretory organs.

It seems probable that the milk of an animal whose rapidity of growth approaches that of the child will be most suitable. The ass, the horse, and the goat are the three domestic animals nearest the child in rate of growth, and some practitioners have been led by experience to prefer the milk of those animals to that of the cow for artificail feeding. As regards phosphocarnic acid, according to Wittmaack, goat's milk closely resembles human.

Lahse, a pupil of Siegfried has found that sterilisation of milk by heat, destroys substantial quantities of phosphocarnic acid, and thus serious injury is done to the food. Sterilisation has been said to destroy the antiscorbutic power of milk, and, if Lahse's observation be a correct one as it appears to be from the results of examination of condensed milk which I have made, then it must, in all probability, also diminish its antirachitic power.

Alongside of this may be placed the observations of Rodet, who states that young dogs fed on raw milk or milk boiled for not longer than twenty minutes increase in weight far more quickly and have a much better nourished appearnace than dogs of the same litter fed on milk boiled for a long time.

In an examination of a condensed milk (Nestle's Milkmaid Brand) I found in 100 grams.

Nitrogenous subs.	7.82	grams
Fats	15.55	"
Phosphocarnic Acid	0.04	"
Total Phosphorus	0.759	"
Inorganic "	0.301	"

There is much added carbohydrate. With the solution which is necessary to bring it to a proper consistence the amounts of caseinogen and of fat are diminished to less than exist in a similar quantity of fresh human milk, the phosphocarnic acid is reduced almost to a vanishing point, and the feeble digestive powers of the young child are burdened with a food much less digestible than the natural food of the suckling.

In a very large proportion of cases, the cause of the enormous death-rate of children in the first year of extrauterine life must be sought for in the nature of the food provided, and the facts brought in this essay lay emphasis on this, that all mothers, so far as health permits, should nourish their children themselves.

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